

Patent Claims

1. A conditionally inducible site-directed mutant cell, comprising
 - a) a mutated allele of a gene; wherein said allele comprises a mutation that was introduced by using a suitable mutagenesis technique,
 - b) a rescue allele of said mutated gene that can be conditionally inactivated,wherein said mutation in said mutated allele of said gene interferes with survival and/or causes an adverse phenotype.
2. The conditionally inducible site-directed mutant cell according to claim 1, wherein said mutated allele of said gene comprises a mutation at the exon or sub-exon level, such as a deletion, point mutation, insertion, inversion, and the like.
3. The conditionally inducible site-directed mutant cell according to claim 1 or 2, wherein said rescue allele and/or its transcription product(s) comprises recombination target sites, e.g. lox or FRT sites, sites for the attachment of antisense oligonucleotides, e.g. DNA, PNA and/or RNA-oligonucleotides, sites for ribozyme activities, and or sites that interfere with specific siRNA for expression.
4. The conditionally inducible site-directed mutant cell according to claim 1 or 2, wherein said rescue allele comprises a conditionally inducible genetic construct which either directly or via its expression product inhibits the function of any non-mutated copy of said mutated allele.
5. The conditionally inducible site-directed mutant cell according to any of claims 1 to 4, containing multiple mutated alleles of genes and/or a multiply mutated allele of a gene together with their suitable rescue allele(s).
6. The conditionally inducible site-directed mutant cell according to any of claims 1 to 5, wherein said allele encodes for titin.
7. The conditionally inducible site-directed mutant cell according to any of claims 1 to 6, wherein said interference with survival and/or adverse phenotype is selected from temporal and/or local phenotypes, such as cell cycle-specific, cell-type specific, tissue-specific,

protein-expression specific, tissue-development specific, organ-specific, organ-development-specific and/or embryonic lethal phenotypes.

8. The conditionally inducible site-directed mutant cell according to any of claims 1 to 7, which is selected from a prokaryotic cell, a eukaryotic cell, a diploid cell, a plant cell, a mammalian cell, a nematode cell, a fish cell, an insect cell, and, in particular, a non-human stem-cell.
9. A conditionally inducible site-directed mutant cell culture, tissue, organ, or non-human embryo, comprising a cell according to any of claims 1 to 8.
10. A conditionally inducible site-directed mutant non-human organism, in particular a genetically deficient or Knock-out-mammal, -rodent, -nematode, -fish, -plant or -insect, comprising a cell according to any of claims 1 to 8 or a culture, tissue or organ according to claim 9.
11. The conditionally inducible site-directed mutant non-human organism according to claim 10, containing multiple mutated alleles of genes and/or a multiply mutated allele of a gene together with their suitable rescue allele(s).
12. The conditionally inducible site-directed mutant non-human organism according to claim 9 or 10, wherein said interference with survival and/or adverse phenotype is selected from temporal and/or local phenotypes, such as cell cycle-specific, cell-type specific, tissue-specific, tissue-development specific, protein-expression specific, organ-specific, organ-development-specific and/or embryonic lethal phenotypes.
13. A method for producing an inducible site-directed mutant cell capable of conditional gene rescue, comprising
 - a) introducing in a target cell a mutated allele of a gene to be mutated by using a suitable mutagenesis technique,
 - b) introducing in said target cell a rescue allele of said gene that can be conditionally in-activated, and
 - c) optionally, cultivating said target cell under conditions that allow for a selection of cells that contain both the mutated allele and the rescue allele of said gene,

wherein said mutation in said mutated allele of said gene interferes with survival and/or causes an adverse phenotype.

14. The method according to claim 13, wherein said suitable mutagenesis technique comprises introducing a mutation at the exon or sub-exon level, such as a deletion, point mutation, insertion, inversion, and the like, preferably by using a suitable mutagenesis technique employing a vector system, irradiation, random integration of foreign DNA, site specific recombination, homologous recombination, and/or chemical mutagenesis.
15. The method according to claim 13 or 14, wherein introducing said rescue allele comprises transfection or infection of the cell with a rescue allele genetic construct comprising recombination target sites, e.g. lox or FRT sites, sites for the attachment of antisense oligonucleotides, e.g. DNA, PNA and/or RNA-oligonucleotides, sites for ribozyme activities, and or sites that interfere with specific siRNA for expression.
16. The method according to claim 13 or 14, wherein introducing said rescue allele comprises transfer of a conditionally inducible genetic construct into the cell, which either directly or via its expression product inhibits the function of any non-mutated copy of said mutated allele.
17. The method according to any of claims 13 to 16, wherein a tissue specific rescue allele and/or mutated allele is introduced.
18. The method according to any of claims 13 to 17, wherein said allele encodes for titin.
19. The method according to any of claims 13 to 18, wherein said cell is selected from a prokaryotic cell, a eukaryotic cell, a diploid cell, a plant cell, a mammalian cell, a fish cell, a nematode cell, an insect cell, and, in particular, a non-human stem-cell.
20. The method according to any of claims 13 to 19, comprising the introduction of multiple mutated alleles of genes and/or a multiply mutated allele of a gene together with their suitable rescue allele(s).

21. The method according to any of claims 13 to 20, wherein said interference with survival and/or adverse phenotype is selected from temporal and/or local phenotypes, such as cell cycle-specific, cell-type specific, tissue-specific, tissue-development specific, organ-specific, organ-development-specific and/or embryonic lethal phenotypes.
22. The method according to any of claims 13 to 20, further comprising
 - d) conditionally inactivating said rescue allele of said gene to be mutated by using a suitable inactivation technique.
23. The method according to claim 22, wherein conditionally inactivating said rescue allele of said gene to be mutated by using a suitable inactivation technique comprises a technique selected from site-directed recombination, such as cre/lox or Flp/FRT inactivation, antisense inactivation using oligonucleotides, e.g. DNA, PNA and/or RNA-oligonucleotides, RNA-interference, such as ribozyme activity inactivation, siRNA expression-inactivation, inactivation of the gene product (protein) and/or its activity and/or inducible inactivation of the non-mutated allele, such as through antibodies, inactivation of the activity of a fusion protein or induced proteolysis.
24. The method according to any of claims 13 to 23, wherein said method is performed in vivo or in vitro.
25. The method according to any of claims 13 to 24, wherein said cell is present in a tissue, organ, non-human embryo or non-human organism, in particular a mammal, rodent, nematode, fish, plant, or insect.
26. A method for the production of an inducible site-directed non-human mutant-organism capable of conditional gene rescue, comprising
 - a) generating an inducible site-directed mutant cell according to the method according to any of claims 13 to 24, and
 - b) generating a non-human mutant organism comprising said mutant cell.
27. An inducible site-directed non-human mutant-organism, produced according to claim 26, in particular a mammal, nematode, rodent, fish, plant, or insect.